Derivation of new human embryonic stem cell lines reveals rapid epigenetic progression in vitro that can be prevented by chemical modification of chromatin.

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Public Summary:
Female cells have two X chromosomes and one X chromosome needs to be silenced for dosage compensation. In this study we examined X chromosome silencing with human embryonic stem cell derivation.

Scientific Abstract:
Human embryonic stem cells (hESCs) are pluripotent cell types derived from the inner cell mass of human blastocysts. Recent data indicate that the majority of established female XX hESC lines have undergone X chromosome inactivation (XCI) prior to differentiation, and XCI of hESCs can be either XIST-dependent (class II) or XIST-independent (class III). XCI of female hESCs precludes the use of XX hESCs as a cell-based model for examining mechanisms of XCI, and will be a challenge for studying X-linked diseases unless strategies are developed to reactivate the inactive X. In order to recover nuclei with two active X chromosomes (class I), we developed a reprogramming strategy by supplementing hESC media with the small molecules sodium butyrate and 3-deazaneplanocin A (DZNep). Our data demonstrate that successful reprogramming can occur from the XIST-dependent class II nuclear state but not class III nuclear state. To determine whether these small molecules prevent XCI, we derived six new hESC lines under normoxic conditions (UCLA1-UCLA6). We show that class I nuclei are present within the first 20 passages of hESC derivation prior to cryopreservation, and that supplementation with either sodium butyrate or DZNep preserve class I nuclei in the self-renewing state. Together, our data demonstrate that self-renewal and survival of class I nuclei are compatible with normoxic hESC derivation, and that chemical supplementation after derivation provides a strategy to prevent epigenetic progression and retain nuclei with two active X chromosomes in the self-renewing state.